

Patent
Case No. GY20a



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group: 1202
Examiner: C. Shen
Applicant: Zahler et al.
Serial No. 763,033
Filed: September 20, 1991
For: Hydroxymethyl(Methylenecyclopentyl)Purines
And Pyrimidines

TH/CHM
10-23-92

Princeton, New Jersey 08543-4000

October 9, 1992

DECLARATION UNDER 37 CFR 1.131

To the Commissioner of Patents and Trademarks:

We, Robert Zahler and William A. Slusarchyk, declare as follows:

1. That we are the inventors of United States patent application Serial No. 763,033 filed September 20, 1991 which is a continuation-in-part of Serial No. 599,568 filed October 18, 1990.

2. That the invention described in Claims 4 - 8, 15, and 23 - 26 of Serial No. 763,033 was conceived and reduced to practice by us in the United States prior to May 2, 1990.

3. That prior to May 2, 1990 the synthesis of [1S-(1 α ,3 α ,4 β)]-2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one was carried out under the direct supervision of William A. Slusarchyk in the United States and reported to Robert Zahler. This compound was assigned the identification number SQ34,676. The synthetic procedure employed is shown in attachment A which is a contemporaneous document prepared by the chemist who performed the synthesis with the dates deleted.

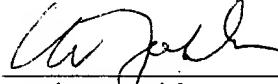
4. That prior to May 2, 1990, samples of SQ34,676 were submitted by Robert Zahler and William A. Slusarchyk for

antiviral testing by the Virology Department of the Squibb Institute.

5. That such antiviral testing was performed in the United States and the results were reported back to Robert Zahler and William A. Slusarchyk prior to May 2, 1990. That the results of such testing are shown in Attachments B through J which are contemporaneous documents prepared by the person who conducted the tests with the date of testing deleted.

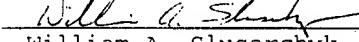
The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of application Serial No. 763,033 or any patent issued thereon.

Date Oct 9, 1992



Robert Zahler

Date October 9, 1992



William A. Slusarchyk

ATTACHMENT A

| | | |
|---|---------|-------------|
| SQUIBB INSTITUTE CHEMICAL TRANSMISSION RECORD Chemistry/Infectious & Metabolic Diseases | Date | Number |
| | Project | SQ-34676 |
| | AVR-000 | Batch NN001 |

SQ-34676 [1S-(1α,3α,4β)]-2-Amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one.

C12H15N5O3

MW/FW 277.28

Physical State: solid

CHIRAL

HANDLING PRECAUTIONS

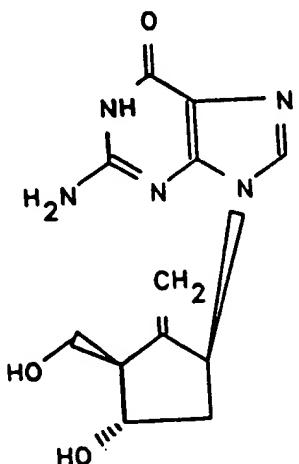
Hygroscopic: yes

Stability: refrigerate

Other:

SOLUBILITY

H2O: %, pH See comments
Adjustable to pH



Novel Compound: yes

Chemist: W. G. YOUNG

MGY

Notebook: L030-195-32

Checked by:

W.A. Slusarchyk

Preliminary Data Sheet:

Complete Data Sheet:

- Comments: 1) 80 mg sent to Virology on .
2) Not soluble in PBS buffer pH 7.2 at 5mg/ml but soluble at 0.145 mg/ml.
3) Soluble in DMSO 5 mg/ml.

Assays:

Antiviral

| | | |
|------------------------|---|-----------------|
| Elemental Analysis (%) | m.p.: > 220°C | IR: 75180 (KBr) |
| C12H15N5O3 · 0.9 H2O | 1H NMR: L030195-32 (DMSO-d6) 270 MHz | |
| Calc. 49.12 | 13C NMR: | |
| H 5.77 | M.S. # MGY 131 | |
| N 23.87 | U.V. λmax at pH 7.2 253.3nm (ε = 12,900) [α]D ^{22°} = +34.0 [c=0.30, water] | |

Fischer:

TLC: Preliminary HI = 97.3 CHCl₃: CH₃OH: NH₃ (6:3:1)

HPLC:
TLE:

sent to Research Chemicals
1 mg Distribution on:
Distribution by RCD:
Microbiology:
Pharmacology:
.....

Copies to:

RIC
RCD
Dr. G. Bisacchi
Dr. C. Cimorusti
Dr. A. Field
Dr. O. Kacy
Dr. W. Koster

Dr. K. Lindner
Dr. C. Moyers
Dr. W. Scott
Dr. R. Zahler

CHEMIST'S REPORT-FLOW SHEET

PAGE 1 of 1

OUTLINE OF PREPARATION OF:

SYNTHETIC CHEMICAL NUMBER
SQ 34,676

DATE

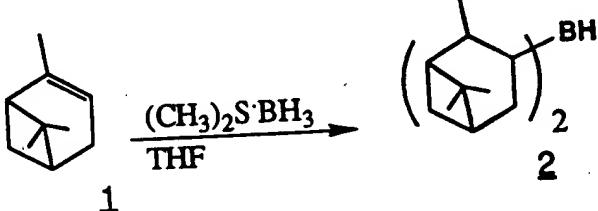
NOTEBOOK
PAGES

YIELDS

CHEMIST
NO.

COMPOUNDS: Start with preparation of first intermediate not previously prepared in this series.
Yields should be calculated on basis of total starting material.

Ref. 1



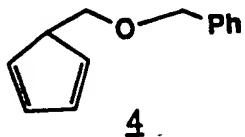
L030081

72%



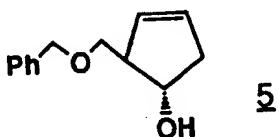
1. Na, THF, -5°
2. Benzyl chloromethyl ether, -50°

Ref. 2



1. 2, -60° for 1 hr,
-10° for 18 hr
2. 3N NaOH, 30% H₂O₂

Ref. 2



L030093

25% for
2 steps

$\text{VO}(\text{acac})_2, \text{t-BuOOH}$
 CH_2Cl_2

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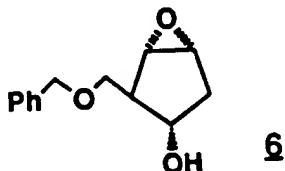
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SYNTHETIC CHEMICAL NUMBER:
SQ 34,676

DATE

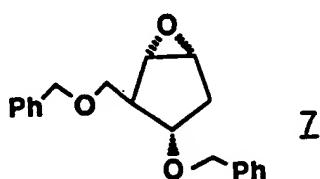
NOTEBOOK
PAGES

YIELDS

CHEMIST
NO.COMPOUNDS: Start with preparation of first intermediate not previously prepared in this series.
Yields should be calculated on basis of total starting material.

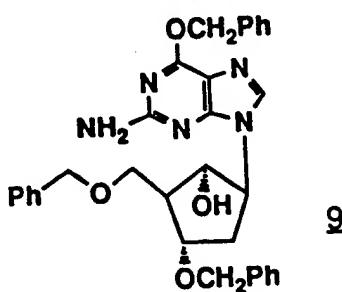
L030105

84%

 $\downarrow \text{Ph CH}_2\text{Br, NaH, DMF}$
 Bu_4NI


L030114

76%

 $\downarrow \text{O-benzylguanine (8), LiH, DMF}$


L030121

60%

 $\downarrow \text{p-Anisylchlorodiphenylmethane, triethylamine, dimethylaminopyridine, CH}_2\text{Cl}_2$

CHEMIST'S NAME

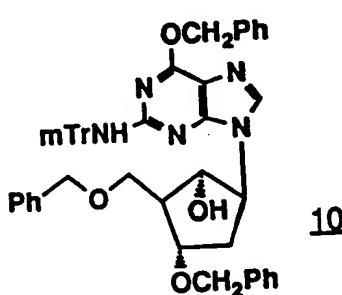
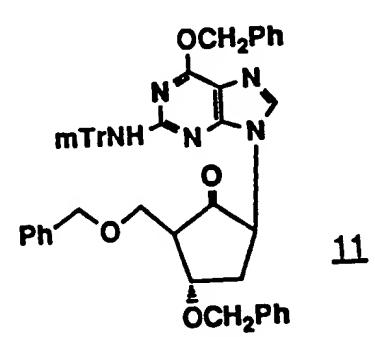
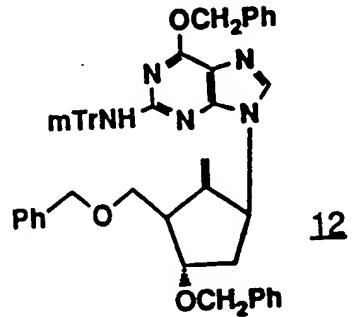
Marian Young

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OUTLINE OF PREPARATION OF:

SYNTHETIC CHEMICAL NUMBER
SQ 34,676

| CHEMIST NO. | COMPOUNDS: Start with preparation of first intermediate not previously prepared in this series. Yields should be calculated on basis of total starting material. | DATE | NOTEBOOK PAGES | YIELDS |
|-------------|--|---------|--------------------------------|--------|
| |  <p style="text-align: center;">10</p> <p style="text-align: center;">DCC, DMSO, Methyl phosphonic acid</p>  <p style="text-align: center;">11</p> | L030167 | | 74% |
| Ref. 3 |  <p style="text-align: center;">12</p> <p style="text-align: center;">Zn, TiCl₄, CH₂Br₂ THF, CH₂Cl₂</p> | L030170 | | |
| | | L030189 | CHEMIST'S NAME Marian Young | |

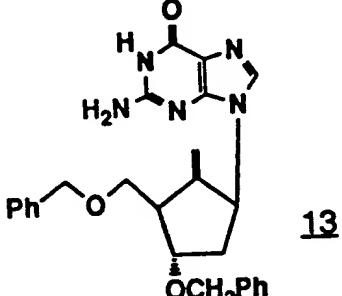
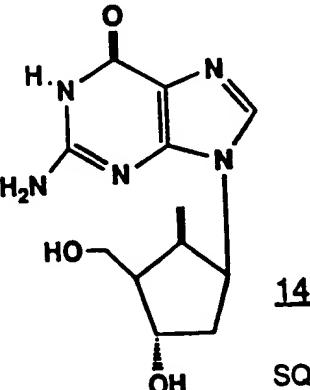
CHEMIST'S REPORT-FLOW SHEET

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OUTLINE OF PREPARATION OF:

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DATE

| CHEMIST NO. | COMPOUNDS: Start with preparation of first intermediate not previously prepared in this series. Yields should be calculated on basis of total starting material. | NOTEBOOK PAGES | YIELDS |
|-------------|--|----------------|-----------------|
| | <p style="text-align: center;">↓</p> <p>1. aqueous 3N HCl, THF, CH₃OH</p> <p>2. 1N KOH to pH 7</p>  <p style="text-align: center;">13</p> | L030190 | 23% for 3 steps |
| | <p style="text-align: center;">↓</p> <p>BCl₃, CH₂Cl₂ -78° to -40°</p>  <p style="text-align: center;">14</p> <p style="text-align: right;">SQ 34,676</p> | L030195 | 62% |

References

1. H. C. Brown, et. al. JOC, 1984, 49, 945
2. K. Biggadike, et. al. J. Chem. Soc. Perkin Trans. 1988, 549
- 3b. L. Lombardo, Tet. Let. 1982, 23, 4293
- 3a. S. Ahmed, Status Report June 5, 1989- November 30, 1989

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Marian Young

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Compound 2

To a solution of 10M borane-methylsulfide (100 ml, 1 mol) in THF (1 l, distilled from K) at 0° was added with stirring 1R (+)- α -pinene (158.8 ml, 1 mol) with an optical purity of less than 90%. The reaction was then placed in the cold room for 16 hr with no stirring. Then more 1R (+)- α -pinene (158.8 ml, 1 mol) was added. A precipitate began forming after 20 min and the suspension was stirred for 8 hrs at 0°. The suspension was allowed to settle for 15 min and the solvents were cannulated away. The solid was washed with ether (3x130 ml) and then dried for 16 hrs on the vacuum pump. The solid was transferred (under N₂ in a dry bag) to dry bottles and stored at -20°. Total 2 obtained was 205 g (72% yield).

Compound 3

Dicyclopentadiene (300 ml) was cracked at 186° under N₂. The cyclopentadiene was distilled through an 18 inch Vigreux column. A total of 110.73 g (b.p. 38°) was collected and stored at -78°.

Compound 5

Cyclopentadiene (28.68 g, 0.434 mol) was warmed from -78° to -30° and cannulated to an addition funnel at -30° under N₂. This was added to 40% Na sand in oil (22.5 g, 0.391 mol) in THF (156 ml, distilled from K) over a 1 hr period keeping the temperature of the reaction at -10°. The cyclopentadienyl sodium solution was then cannulated to an addition funnel at 0° over 1.3 hr. This solution was added to benzylchloromethyl ether (65.19 ml, 0.469 mol) in THF (130 ml) at -50° over 1.3 hr.

This suspension was stirred 1.3 hr at -45° and then cooled to -60°. The suspension was diluted with THF (390 ml) and then compound 2 (136 g, 0.477 mol) was added as a solid under a N₂ atmosphere. The reaction was then stirred 1 hr at -60° and warmed to -10° over 1.5 hr. This was stirred at that temperature for 16 hr. The reaction was concentrated to 1/2 the volume *in vacuo*, and the slurry was diluted with 390 ml of ether. The reaction was cooled to 0° and 3N NaOH (156 ml, 0.469 mol) was added over 45 min keeping the temperature at 0°. Then 30% H₂O₂ (156 ml) was added over 1 hr keeping the temperature below 12°. The reaction was stirred 1 hr at 10°.

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The layers were separated, and the water layer was washed with ether (300 ml). The ether layers were combined, washed with brine (200 ml), dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified on Merck silica gel (5 l packed in petroleum ether:ether, 2:1). The column was eluted with petroleum ether:ether (2:1 to 1:1) to give 20 g (25% yield) of 5.

Compound 6

To a solution of compound 5 (29.63 g, 0.145 mol) and vanadyl acetylacetone (400 mg) in dichloromethane (60 ml, distilled from CaH₂) under N₂ was added 3M t-butyl hydroperoxide (87 ml, 0.261 mol) in 2,2,4-trimethylpentane (87 ml, 0.261 mol) over 75 min at such a rate to keep the temperature at 25° with a water bath. After stirring 16 hr at 25° the reaction was cooled to 0° and saturated aqueous sodium sulfite (150 ml) was added over 1 hr keeping the reaction temperature below 20°. The reaction was stirred for 1.5 hr at room temperature. The layers were separated, and the aqueous layer was extracted with 50 ml of dichloromethane. The organic layers were combined, washed with water (50 ml), dried over sodium sulfate, filtered and concentrated *in vacuo*.

The residue was purified on Merck silica gel (2 l, petroleum ether: ether 1:1). The column was eluted with petroleum ether:ether (2:1) to give 24.19 g of pure 6. Fractions containing impure 6 were purified on Merck silica gel (400 ml, petroleum ether:ether 1:1). The column was eluted with petroleum ether:ether (1:1) to give 2.71 g of pure 6. Total yield of 6 was 26.90 g (84%).

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L030195-32**Compound 7**

To a suspension of 60% sodium hydride in mineral oil (5.11 g, 0.128 mol) in tetrahydrofuran (247 ml) under N₂ was added compound 6 (25.58 g, 0.116 mol) in tetrahydrofuran (123 ml) dropwise over 20 min at room temperature. This was stirred with an overhead stirrer for 2 hr at room temperature and 1 hr at 40°. The reaction was cooled to room temperature, and benzyl bromide (15.2 ml, 0.128 mol) and tetrabutyl ammonium iodide (412 mg) were added. After 3 hr ethanol (20 ml) was added, and the reaction was stirred for 10 min. The solvents were removed *in vacuo*. The residue was partitioned between water (200 ml) and ether (200 ml). The water layer was extracted with ether (200 ml) and the organic layers were combined, dried over sodium sulfate, filtered and concentrated *in vacuo*.

The residue was purified on Merck silica gel (2 l, petroleum ether:ether 3:1). Elution with a gradient of petroleum ether:ether (3:1 to 1:1) gave 27.21 g of 7 (76% yield).

Compound 9

To a solution of 7 (6.20 g, 20 mmol) and O-benzylguanine 8 (9.64 g, 40 mmol, dried 50° *in vacuo*) in dry dimethyl formamide (80 ml, over sieves) at 60° under N₂ was added lithium hydride (80 mg, 10 mmol). The temperature was raised to 125° and stirred for 10 hr and then lowered to room temperature and stirred for 6 hr. Acetic acid (572 µl, 10 mmol) was added and the reaction was stirred for 10 min. The solvents were removed *in vacuo*, and the residue was purified on Merck silica gel (2 l, dichloromethane). The column was eluted with a gradient of dichloromethane to dichloromethane:methanol (95:5) to give 9.03 g of impure 9. This was purified on SilicAR CC-7 (1 l, chloroform) and eluted with a gradient of chloroform to chloroform:ethanol (88:12) to give 6.63 g (60% yield) of 9.

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Compound 10

To a solution of compound 9 (5.45 g, 9.89 mmol) in dichloromethane (75 ml, distilled from CaH₂) under N₂ was added p-anisylchlorodiphenylmethane (3.37 g, 10.93 mmol), triethylamine (2.35 ml, 16.81 mmol) and dimethylaminopyridine (40 mg). The reaction was stirred at room temperature for 3 hr and was then extracted with 5% sodium bicarbonate (30 ml) and water (10 ml). The organic layer was dried, filtered and concentrated *in vacuo*. The residue was purified on SilicAR CC-7 (600 ml packed in chloroform). The column was eluted with chloroform:ethanol (99:1) to give 1.5 g of pure 10. Fractions containing impure 10 were purified on SilicAR CC-7 (700 ml packed in chloroform). The column was eluted with chloroform:ethanol (99.5:0.5) to give 4.54 g of 10. Total yield of 10 was 6.04 g (74%).

Compound 11

To a solution of 10 (4.10 g, 4.88 mmol, dried by concentration from toluene) in dimethyl sulfoxide (12 ml, dried over sieves) was added dicyclohexylcarbodiimide (3.08 g, 14.9 mmol) and methyl phosphonic acid (0.239 g, 2.49 mmol). The reaction was stirred 4 hr at room temperature and then sat for 16 hr at -20°. The reaction was then warmed to room temperature and oxalic acid dihydrate (60 mg) in methanol (8.0 ml) was added. This was stirred for 2.5 hr. The reaction was filtered, and the filtrate was dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 ml), filtered and concentrated *in vacuo*. The NMR spectra indicated there was unreacted dicyclohexylcarbodiimide in the residue.

The residue was dissolved in dimethylsulfoxide (9 ml) and then methyl phosphonic acid (150 mg) in methanol (6 ml) and oxalic acid dihydrate (60 mg) were added. This was stirred for 4 hrs. The reaction was filtered and the precipitate was washed with dichloromethane (120 ml). The organic layer was washed with water (3 x 50 ml), dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 ml) filtered and concentrated *in vacuo* to give 3.73 g of 11. The NMR spectra indicated there was no unreacted dicyclohexylcarbodiimide in the residue.

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Compound 12

To a solution of 11 (1.8 g, 2.19 mmol) in dichloromethane (40 ml distilled from CaH₂) was added a slurry of 0.3M Zn-TiCl₄-CH₂Br₂ (40 ml, 12.3 mmol) by teflon cannula under N₂. After 3 hrs the reaction was poured slowly into saturated sodium bicarbonate (200 ml) and dichloromethane (200 ml). The mixture was filtered through celite and the celite pad was washed with dichloromethane (3 x 75 ml). The organic layers were combined, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 ml) and refiltered through celite. The pad was washed with dichloromethane (30 ml). The organic washes were combined and concentrated to give 1.43 g of 12.

Compound 13

To a solution of 12 (2.5 g, crude) in tetrahydrofuran (25 ml) and methanol (25 ml) was added 3N HCl (12.5 ml). The reaction was heated at 50° for 2.5 hr and then cooled to room temperature. The pH of the reaction was raised to 7.3 with 1N KOH, and the mixture was extracted with ethyl acetate (3 x 120 ml). The extracts were combined, dried over sodium sulfate, filtered and concentrated *in vacuo*.

The residue was purified on Merck silica gel (340 ml, packed in chloroform: ethanol, 97:3). The column was eluted with a gradient of chloroform:ethanol (97:3 to 80:20) to give 316 mg (23% yield for 3 steps) of compound 13.

Compound 14

To a solution of 13 (304 mg, 0.673 mmol) in dichloromethane (12 ml, distilled from CaH₂) at -78° under N₂ was added 1M boron trichloride in dichloromethane (6.7 ml, 6.7 mmol). The reaction was stirred at -78° for 2 hr and -40° for 30 min. It was then cooled to -78° and methanol (60 ml) was added slowly over 10 min. The reaction mixture was concentrated from methanol (4 x 40 ml). After dissolving the reaction in methanol (5 ml) and water (5 ml), the pH was adjusted to 6.8 with 1N KOH. The slurry was concentrated *in vacuo*, suspended in water and purified on CHP-20P (16 ml, water). The column was eluted with a gradient of water to water:acetonitrile (93:7) to give 115 mg (62% yield) of 14 as a solid, m.p. >220°.

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Analysis: Calc'd for $C_{12}H_{15}N_5O_3 \cdot 0.9 H_2O$

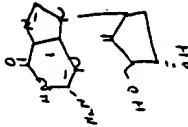
C, 49.12; H, 5.77; N, 23.87

Found: C, 49.17; H, 5.87; N, 23.81

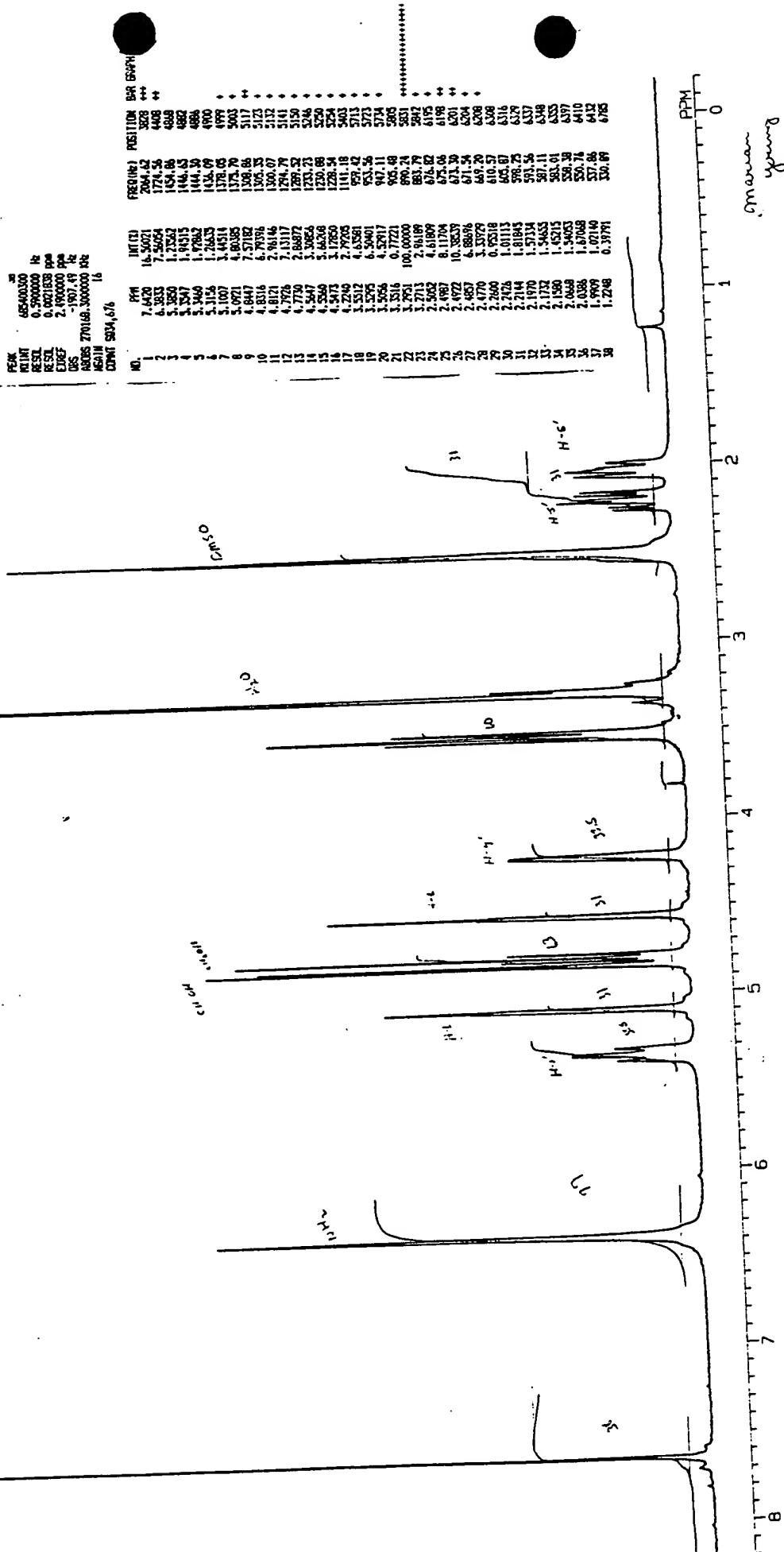
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卷之三

09:41:34
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DEFILE PROTON
COMINT SU34, 676
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BO
SCANS

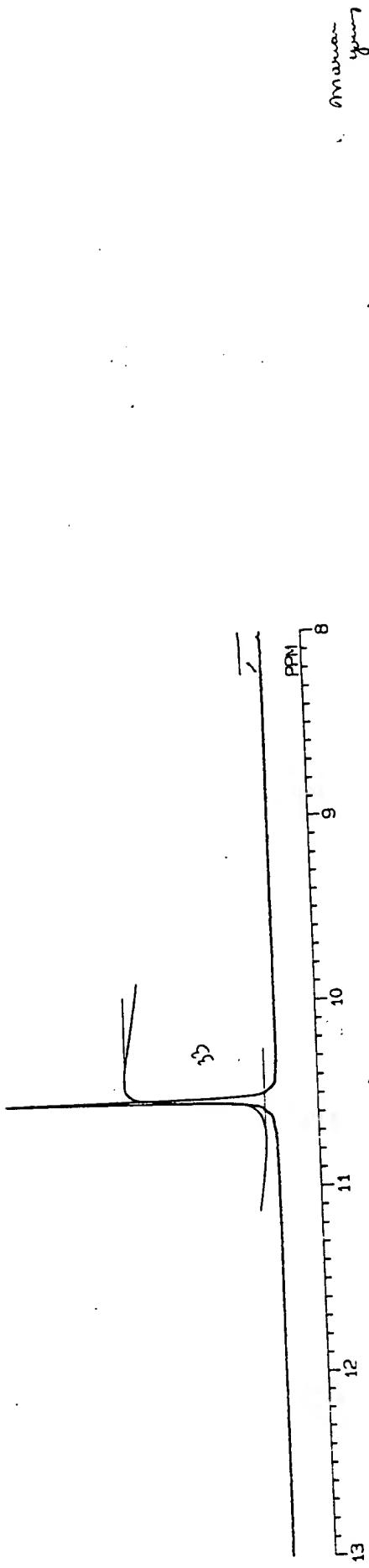


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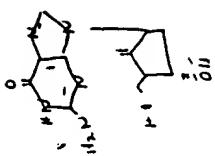
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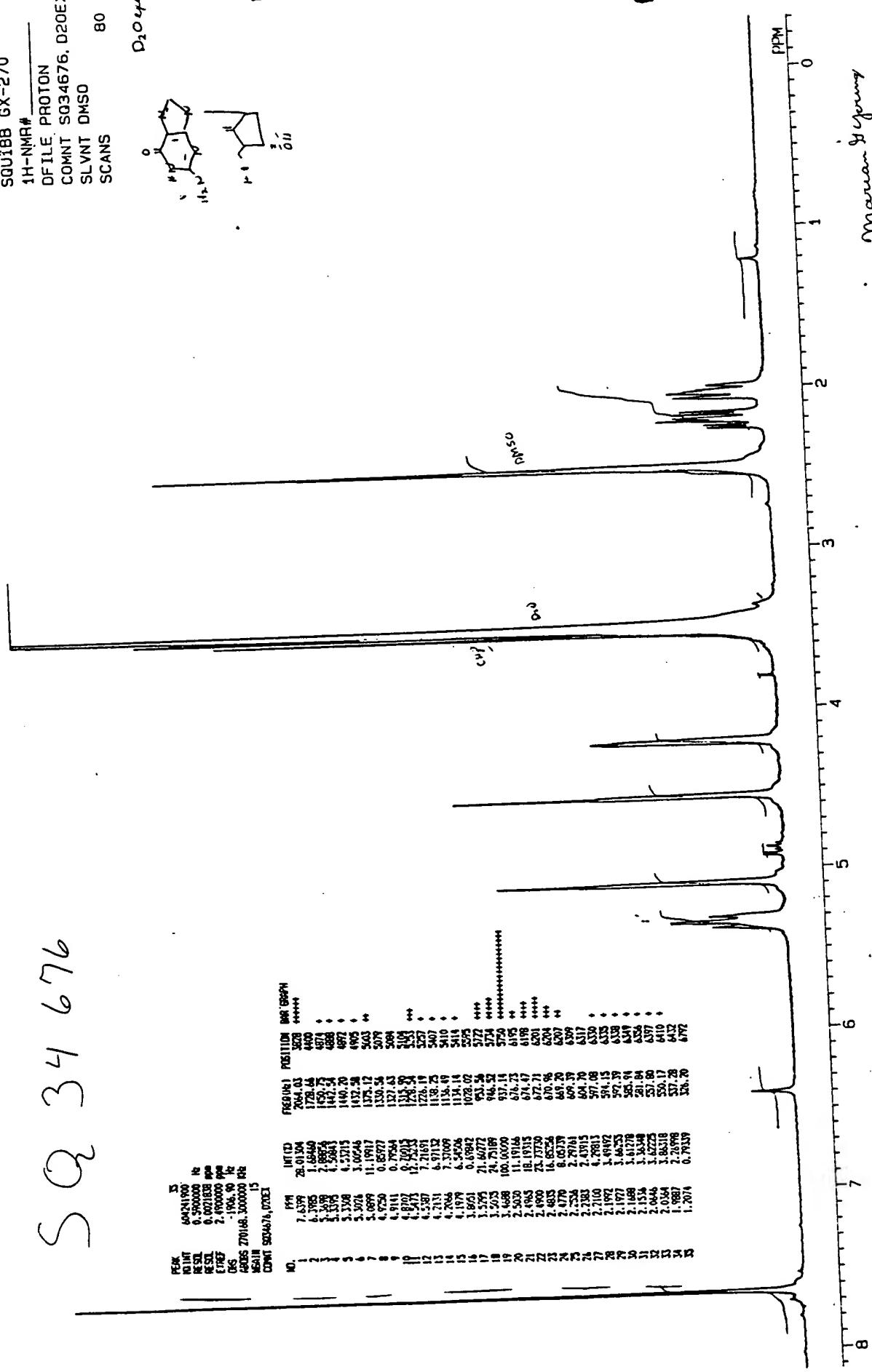


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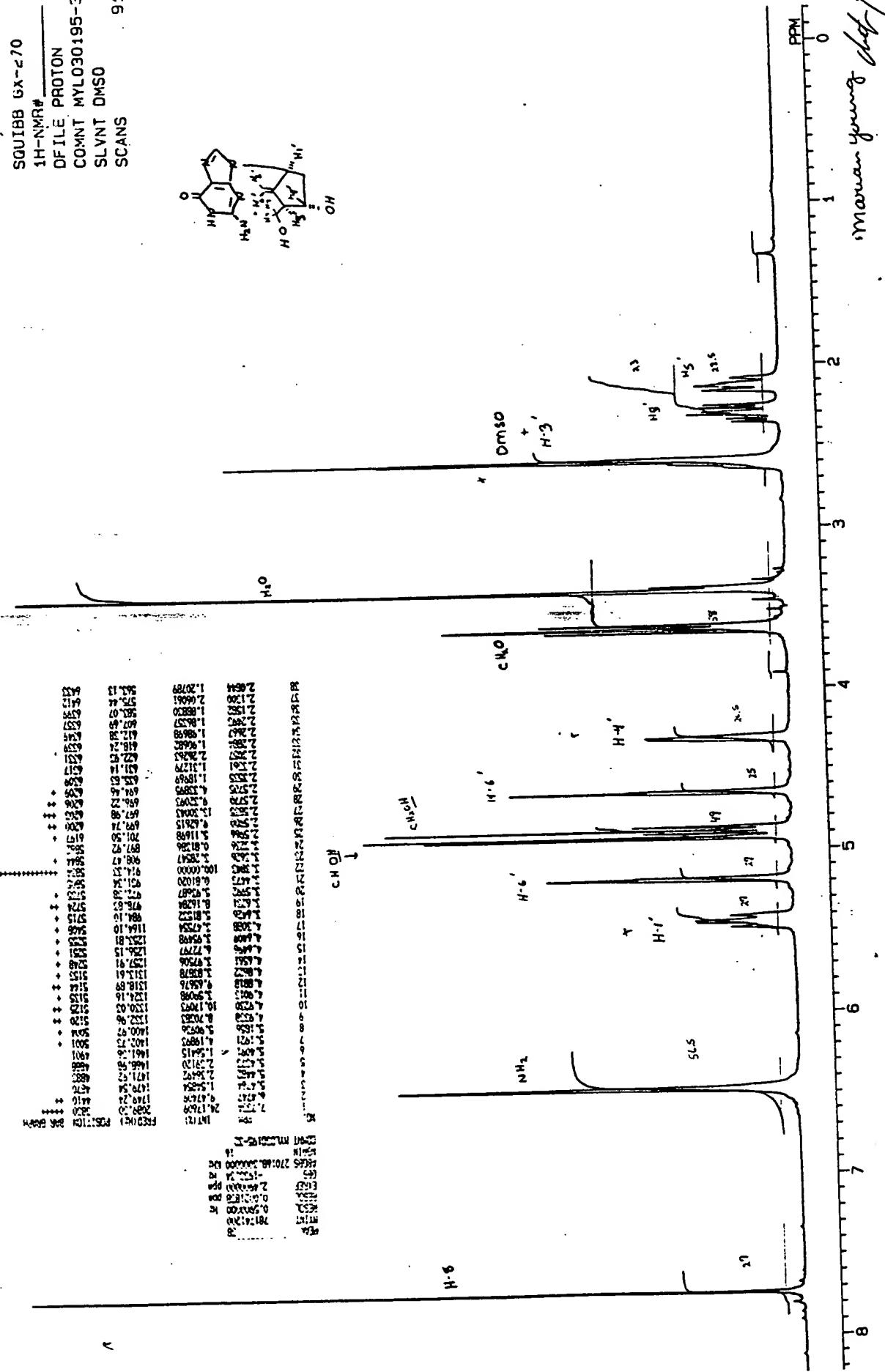
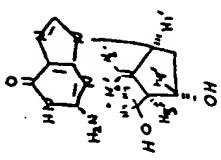
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S Q 34 676



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 SCANS



MYL030195-32

L030-195-32

SQ 34,676

SQUBB GX-179778

13C-NMR#

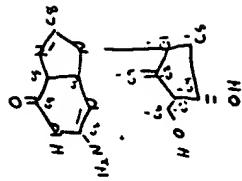
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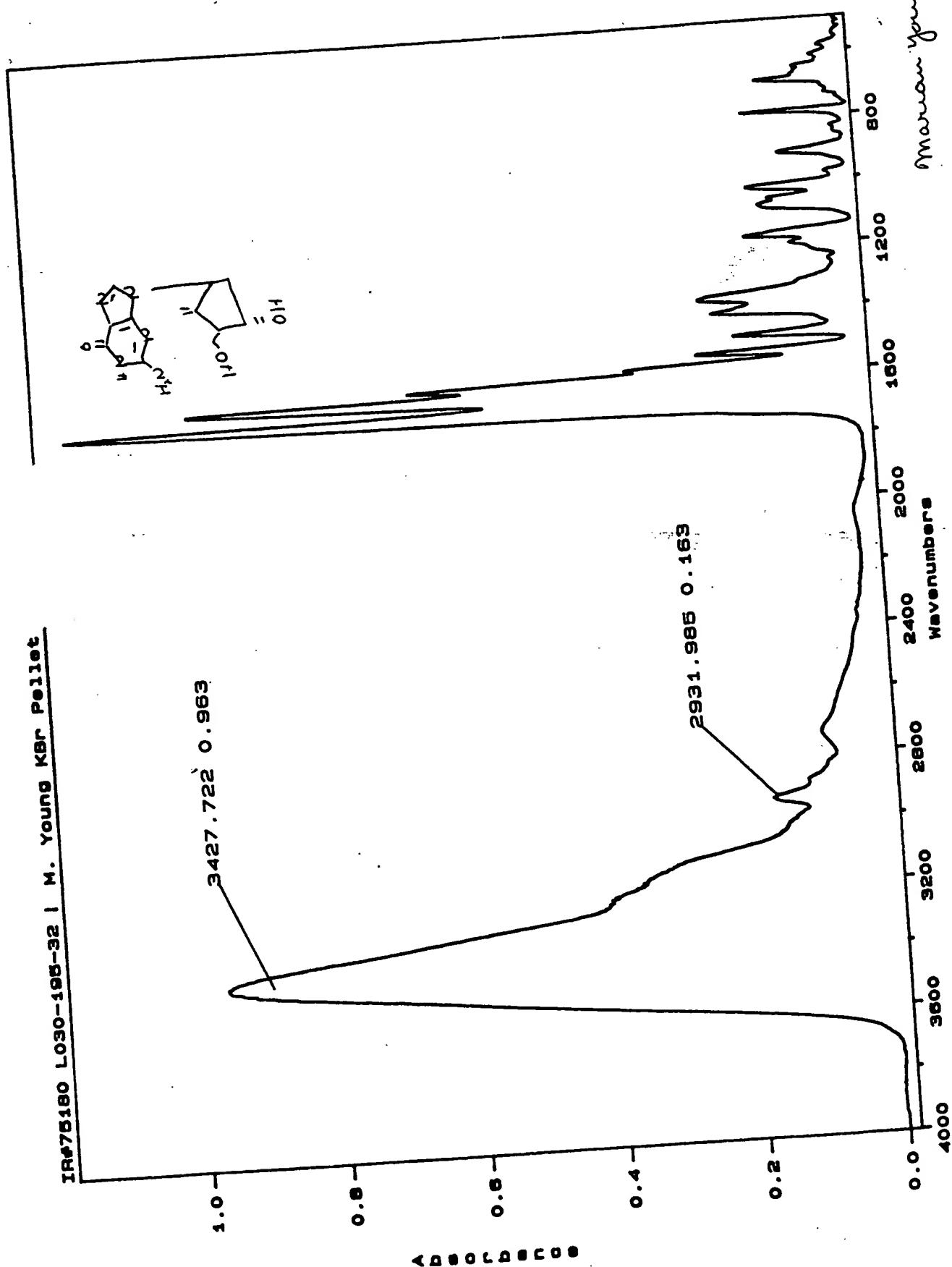
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for water up



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|-------------|---------|-----------------|------------|
| 7 | 71.9400 | H _{4'} | |
| RIAT | 71.9400 | H _{4'} | |
| RESOL | 71.9400 | H _{4'} | |
| E1REF | 71.9400 | H _{4'} | |
| US | 71.9400 | H _{4'} | |
| ADSB | 71.9400 | H _{4'} | |
| HEATH | 71.9400 | H _{4'} | |
| COMNT | 71.9400 | H _{4'} | |
| L030-195-32 | 71.9400 | H _{4'} | |
| | 116.740 | C ₁ | |
| | 116.543 | C ₁ | |
| | 116.345 | C ₁ | |
| | 116.147 | C ₁ | |
| | 115.949 | C ₁ | |
| | 115.751 | C ₁ | |
| | 115.553 | C ₁ | |
| | 115.355 | C ₁ | |
| | 115.157 | C ₁ | |
| | 114.959 | C ₁ | |
| | 114.761 | C ₁ | |
| | 114.563 | C ₁ | |
| | 114.365 | C ₁ | |
| | 114.167 | C ₁ | |
| | 113.969 | C ₁ | |
| | 113.771 | C ₁ | |
| | 113.573 | C ₁ | |
| | 113.375 | C ₁ | |
| | 113.177 | C ₁ | |
| | 112.979 | C ₁ | |
| | 112.781 | C ₁ | |
| | 112.583 | C ₁ | |
| | 112.385 | C ₁ | |
| | 112.187 | C ₁ | |
| | 111.989 | C ₁ | |
| | 111.791 | C ₁ | |
| | 111.593 | C ₁ | |
| | 111.395 | C ₁ | |
| | 111.197 | C ₁ | |
| | 110.999 | C ₁ | |
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| | 110.395 | C ₁ | |
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| | 109.999 | C ₁ | |
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| | 108.999 | C ₁ | |
| | 108.791 | C ₁ | |
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| | 108.197 | C ₁ | |
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| | 103.593 | C ₁ | |
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| | 82.197 | C ₁ | |
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| | 58.593 | C ₁ | |
| | 58.395 | C ₁ | |
| | 58.197 | C ₁ | |
| | 57.999 | C ₁ | |
| | 57.791 | C ₁ | |
| | 57.593 | C ₁ | |
| | 57.395 | C ₁ | |
| | 57 | | |

SG 341676

IR#75180 L030-195-32 | M. Young KBr Pellet



| Peak | Pick | Intensity |
|------------------|------|-----------|
| cm ⁻¹ | | |
| 644. | .265 | 0.063 |
| 655. | .839 | 0.082 |
| 684. | .773 | 0.118 |
| 783. | .148 | 0.143 |
| 812. | .388 | 0.082 |
| 1018. | .550 | 0.143 |
| 1053. | .200 | 0.112 |
| 1072. | .489 | 0.126 |
| 1170. | .865 | 0.150 |
| 1185. | .841 | 0.085 |
| 1363. | .759 | 0.224 |
| 1408. | .125 | 0.207 |
| 1481. | .424 | 0.176 |
| 1535. | .434 | 0.292 |
| 1570. | .155 | 0.336 |
| 1597. | .180 | 0.650 |
| 1629. | .952 | 0.970 |
| 1683. | .963 | 1.148 |
| 2879. | .904 | 0.112 |
| 2931. | .885 | 0.163 |
| 3211. | .881 | 0.398 |
| 3219. | .397 | 0.404 |
| 3232. | .900 | 0.408 |
| 3427. | .722 | 0.963 |

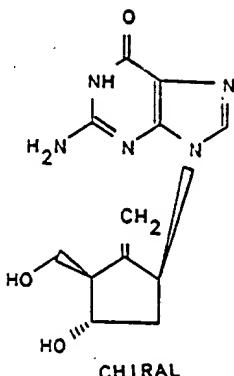
Marrow ground

ATTACHMENT B

PRELIMINARY

IN VITRO ANTIVIRAL ACTIVITY CMVCompound: 50 34676 FW 277.28

Structure:

Concentrations: 25 mg/mlSolvent: DMSOAssay Procedure: PLAQUE REDUCTION IN WI-38 CELLSCompound (μ M)ED₅₀ CMV, 36-36.1Control (μ M) AD169 SQ 31917 (DHPG)ED₅₀ CMV: 2-4Comments:

| | CONC | | % REDUCTION |
|--|---------|-------|-------------|
| | μ M | ug/ml | |
| | 36.1 | 100 | 100 |
| | 36.1 | 10 | 57 |
| | 3.6.1 | 1 | 36 |

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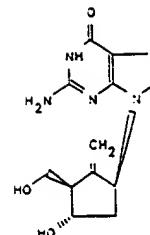
Assay Date: _____

Analyst: A.V. TuomariB. McGeever-Rubin

* TO BE REPEATED

ATTACHMENT CIN VITRO ANTIVIRAL ACTIVITYCompound: SQ 34676

Structure:

Concentrations: 20 mg/mlSolvent: DMSOAssay Procedure: PLAQUE REDUCTION IN WI-38 CELLSCompound (μ M)

| | | | | |
|---|----------------|---|----------------|-------------------------|
| <u>ED₅₀ HSV-1: (SCH)</u> | <u><3.6</u> | <u>ED₅₀ HSV-2: (180)</u> | <u><3.6</u> | Toxicity: <u>NT 361</u> |
|---|----------------|---|----------------|-------------------------|

Control (μ M)

| | | | |
|---|------------------|---|-------------|
| <u>ED₅₀ HSV-1: (SCH)</u> | <u>0.22-0.44</u> | <u>ED₅₀ HSV-2: (180)</u> | <u>0.22</u> |
|---|------------------|---|-------------|

Comments:PERCENT REDUCTION OF PLAQUES

| <u>μg/ml</u> | <u>μM</u> | <u>SCH</u> | <u>186</u> |
|-----------------------------|--------------------------|--------------|--------------|
| <u>900</u> | <u>361</u> | <u>100 %</u> | <u>100 %</u> |
| <u>10</u> | <u>3.6</u> | <u>100 %</u> | <u>100 %</u> |
| <u>1</u> | <u>0.36</u> | <u>73 %</u> | <u>55 %</u> |

Notebook Page: L 300 ; 063-065

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 Dr. S. Ahmad

Assay Date:

Analyst: B McGeever
AV Tuomari

X preliminary results

ATTACHMENT D

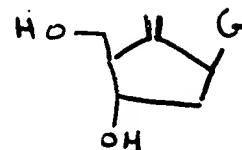
CMV

IN VITRO ANTIVIRAL ACTIVITY

Compound: SQ 39676

Structure:

Concentrations: 50 mg/ml



Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μM)

ED_{50} CMV: 90
(AD 169)

Control (μM): SQ 31919

ED_{50} CMV: 0.8 - 2
(AD 169)

Comments:

| μM | CONC $\mu g/ml$ | % REDUCTION | |
|---------|--------------------|-------------|--|
| | | | |
| 361 | 100 | 72 | |
| 180 | 50 | 65 | |
| 90 | 25 | 47 | |
| 36.1 | 10 | 6 | |
| 18 | 5 | 19 | |

Notebook Page: L 374-098

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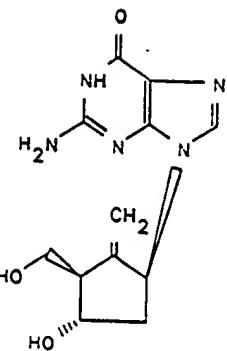
Analyst: A.V. Tuomari,
B. McGeever-Rubin

ATTACHMENT E

VZV

IN VITRO ANTIVIRAL ACTIVITY(ELLEN)Compound: SQ 34676 FW 277.28

Structure:

Concentrations: 50 mg/mlSolvent: DMSOAssay Procedure: PLAQUE REDUCTION IN WI-38 CELLSCompound (μM) $\text{ED}_{50}^{\text{VZV}}$ ELLEN : ≤ 7.2 Control (μM) SQ 31933 $\text{ED}_{50}^{\text{VZV}}$ ELLEN : 24 -Comments:

| <u>μM</u> | <u>CONC.</u> | <u>% REDUCTION</u> | |
|---------------------------------|--------------|--------------------|------------|
| | | <u>ug/ml</u> | <u>100</u> |
| <u>361</u> | <u>100</u> | <u>100</u> | |
| <u>180</u> | <u>50</u> | <u>100</u> | |
| <u>90</u> | <u>25</u> | <u>92</u> | |
| <u>36.1</u> | <u>10</u> | <u>87</u> | |
| <u>18</u> | <u>5</u> | <u>69</u> | |
| <u>7.2</u> | <u>2</u> | <u>56</u> | |

Notebook Page: L374-097

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Assay Date:

Analyst: A.V. Tuomari
B. McGeever Rubin

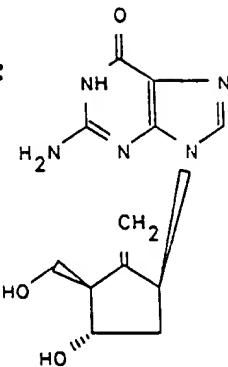
ATTACHMENT F

IN VITRO ANTIVIRAL ACTIVITY

VZV
ELLEN

Compound: SQ 34676

Structure:



Concentrations: 50 mg/ml

Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μM)

ED₅₀ VZV : 19-36
ELLEN

Control (μM) SQ 31933

ED₅₀ VZV : 2-4
ELLEN

Comments:

| CONC. μM | % REDUCTION | |
|-------------|-------------|----|
| | ug/ml | |
| 94 | 25 | 86 |
| 36.1 | 10 | 65 |
| 18.8 | 5 | 30 |
| 7.5 | 2 | 10 |

Notebook Page: L 374 - 105

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Assay Date:

Analyst: B. McGeever Rubin
A.V. Tuomari

ATTACHMENT 6

* VZV
PP II A

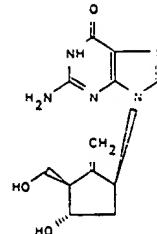
IN VITRO ANTIVIRAL ACTIVITY

Compound: SQ 34676

Structure:

Concentrations: 20 mg/ml

CHIRAL



Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION in WI-38 cells

Compound (μM)

VZV
 ED_{50} : PP II A: 23.6

Control (μM)

VZV
 ED_{50} : PP II A: 31933 (PCV)

VZV
 ED_{50} : PP II A: 0.22-0.44

Comments:

| <u>ug/ml</u> | <u>μM</u> | <u>% Reduction</u> |
|--------------|---------------------------|--------------------|
| 100 | 361 | 100 % |
| 10 | 36 | 100 % |
| 1 | 3.6 | 87 % |

Notebook Page: 1300; 063-065

Copies to:

Assay Date: _____

Analyst: B. McGeehan
A. Tuomari

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* VZV (PP II A) - wild type VZV strain
Sawyer et al., 1988

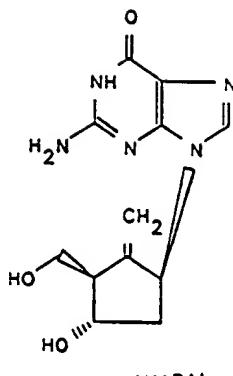
ATTACHMENT H

IN VITRO ANTIVIRAL ACTIVITY HSV

Compound: SQ 34676 FW 277.28

Structure:

Concentrations: 50 mg/l ml



Solvent: DMSO

Assay Procedure: PLAQUE REDUCTION IN WI-38 CELLS

Compound (μ M)

ED₅₀ HSV-1: 3.6 ED₅₀ HSV-2: 7.2 - 18 Toxicity: NT OT 9C
Control (μ M) SQ 31933 (Acv)

Comments:

PERCENT REDUCTION OF PLAQUES

| CONC | SCH | 186 |
|------|-------|-----|
| µM | µg/ml | |
| 90 | 25 | — |
| 36 | 10 | 99 |
| 18 | 5 | 97 |
| 7.2 | 2 | 78 |
| 3.6 | 1 | 51 |
| 1.8 | 0.5 | 24 |
| 0.72 | 0.2 | 13 |

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Assay Date:

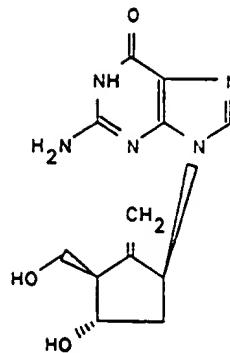
Analyst: A.V.Tuomari

DR. W. SCOTT
DR. A.K. FIELD
DR. W. KOSTER
DR. B. TERRY
DR. R. ZAHLER.
DR. G. BISACCHI
DR. W. SWISCHCZYK
DR. J. TINO
DR. M. HAFFEY
DR. V. GOOD FELLOW
DR. G. YAMANAKA
DR. G. VITE

ATTACHMENT I

CELL GROWTH INHIBITION

CHIRAL

Compound: SQ 34676 Structure:Solvent: DMSO

Assay Procedure: Inhibition of WI-38 cell proliferation after 3 days in the presence of compound

Results (μM):ED₅₀ 450CELL GROWTH INHIBITION AS PERCENT OF CONTROL

| <u>Conc. μM</u> | <u>%</u> |
|---------------------------------------|-------------|
| <u>600</u> | <u>50.0</u> |
| <u>150</u> | <u>37.5</u> |
| <u>38</u> | <u>72.9</u> |
| <u>10</u> | <u>68.0</u> |

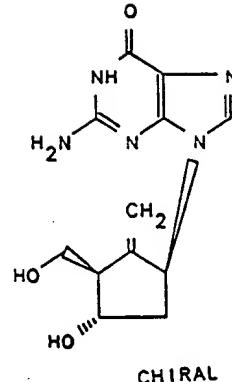
Notebook Page: L799 c 013

Assay Date: -Analyst: P. VetterCopies to: Dr. A. K. Field
Dr. M. L. Haffey
Dr. R. Zahler

ATTACHMENT J

CELL GROWTH INHIBITION

Compound: SQ 34676 NN001 Structure:



Solvent: DMSO

Assay Procedure: Inhibition of WI-38 cell proliferation after 3 days in the presence of compound

Results (μM):

ED₅₀ 310

CELL GROWTH INHIBITION AS PERCENT OF CONTROL
(3 DAYS POST-COMPound)

| <u>Conc. μM</u> | <u>%</u> |
|---------------------------------|------------|
| <u>800</u> | <u>17</u> |
| <u>200</u> | <u>32</u> |
| <u>50</u> | <u>66</u> |
| <u>12.5</u> | <u>106</u> |

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Assay Date: _____

Analyst: A.V. Tuomari

Copies to: Dr. A. K. Field
Dr. M. L. Haffey
Dr. R. Zahler